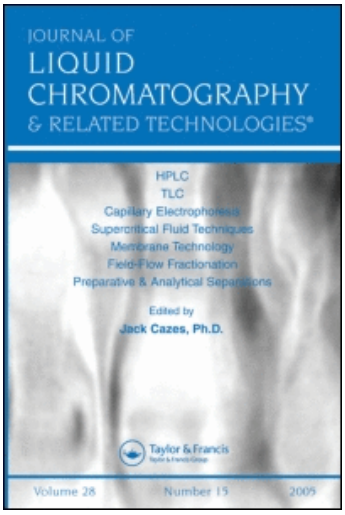

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IMPROVED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) ASSAY METHOD FOR CEFTIZOXIME

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ABSTRACT

We improved a high-performance liquid chromatographic method for the quantitative determination of ceftizoxime in human serum and urine using cefotaxime as internal standard. It employs a μ Bondapak Alkyl Phenyl column, elution with acetonitrile-phosphate buffer and measurement of UV absorption at 254 nm. Results obtained using the HPLC assay were compared to those obtained using a microbiological assay. The correlation coefficient was 0.987 (n:25). The method is rapid, accurate and reproducible with a sensitivity of 2.5 μg/ml of ceftizoxime. Cefotaxime and its major metabolite, the desacetylcefotaxime, can also be quantitated by this procedure.

INTRODUCTION

Ceftizoxime (FK 749, SK & F 88373-Z){sodium (6R, 8R)-7-{(Z)-2-(2-imino-4-thiazolin-4-yl)-2-methoxy-iminoacetamido}-8-oxo-5-thial-azabicyclo {4.2.0} oct-2-ene-2-carboxylate} is a new parenteral third-generation cephalosporin. The structural formula is shown in Figure 1. Suzuki et al. (1) used high-performance liquid chromatography (HPLC) for the analysis of ceftizoxime in rat serum, bile and urine. This method did not use an internal standard and need to be modified to quantitate ceftizoxime in human samples. We report here an improved method for determination of ceftizoxime in human serum and urine, using cefotaxime as an internal standard. The total time needed to complete the analysis of ceftizoxime in individual serum samples is about 20 minutes and the amount of serum required for the assay is 0.5 ml.

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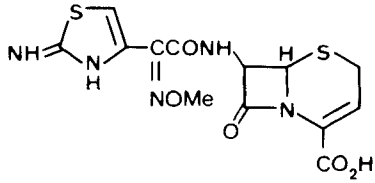

Fig. 1. Chemical structure of ceftizoxime

MATERIALS AND METHODS

Sodium ceftizoxime was obtained from Smith Kline and French Laboratories, Philadelphia, PA (lot number, 81225). Cefotaxime (lot number RP-3717) and desaceytlcefotaxime (lot number RO28628A) were kindly provided by Hoechst-Roussel Pharmaceuticals Inc, Sommerville, NJ. Acetonitrile, potassium phosphate monobasic and phosphoric acid 85%, all HPLC grade, were purchased from Fisher Scientific Co, Pittsburg, PA.

The chromatographic system consisted of a model 6000 A solvent delivery system, a U-6K injector and a variable - wavelength model 450 detector (Waters Associates, Milford, MA.) set at 254 nm and 0.1 A.U.F.S. Separation was accomplished on a μ Bondapak Alkyl Phenyl column (30 cm of length X 4 mm I.D.; Waters Associates, Milford, MA.). A mixture of 13% acetonitrile in 0.02 M (pH 2.6) phosphate buffer was used as the chromatographic eluant, at a flow-rate of 1.5 ml/min (pressures of 1,500 to 2,000 psi). The buffer solution was filtered through an HA 0.45-μ filter (Millipore Corp. Bedford, MA.) and the acetonitrile through a FH 0.45-μ filter (Millipore Corp. Bedford, MA.). The mobile phase was deaerated with an ultrasonic water bath for 20 minutes. The elution profile was recorded on a Perkin-Elmer recorder, (model 023 Perkin-Elmer, Norwalk, CT) using a chart speed of 30 cm/h and was set at 1.0 mV.

A standard solution of sodium ceftizoxime was prepared in HPLC-grade water at a concentration of 1,000 μg/ml. This solution was further diluted ad 10 ml with pooled lyophilized frozen human serum (freshly reconstituted with ten ml of HPLC-grade water) to give concentrations of 160, 120, 80, 60, 30, 20 and 10 μg/ml. These concentrations represent serum concentrations to be expected from the administration of 1 g dose of ceftizoxime (2). The standard solution was freshly prepared each day and kept under refrigeration when not in use. The standard solution of sodium cefotaxime, the internal standard, was prepared similarly.

After adding 75 µl of the internal standard solution (sodium cefotaxime, 1,000 µg/ml) to a 0.5 ml aliquot of serum sample containing ceftizoxime, an equal volume (0.5 ml) of acetonitrile was added. The sample was vortexed for 20 sec at maximum speed, to ensure complete protein precipitation. The mixture was centrifuged at 3,000 rpm for ten minutes. A supernatant aliquot of 10 or 20 µl was injected into the HPLC.

Fresh urine from a healthy volunteer (Clinitest ^{R}, negative) to which graded concentrations of ceftizoxime were added (150, 100, 75, 50, 25, 12.5 and 6.25 µg/ml) was used to construct the urine calibration curve. These urine concentrations are expected from the administration of 1 g dose of ceftizoxime (3).

Unknown urine samples were diluted with HPLC-grade water to a concentration in the range of the standard curve. To 0.5 ml of diluted urine, 75 µl of sodium cefotaxime solution (1,000 µg/ml), as internal reference standard, and 0.1 ml of phosphate buffer-acetonitrile 13% mixture were added. The volume injected varied between 5 to 20 µl.

To compare the HPLC assay and the microbiological assay, we used 25 serum samples from human volunteers who had received a single 1 g dose of ceftizoxime (4), and pooled fresh serum to which known amounts of ceftizoxime were added. The microbiological assay was performed using a disc agar diffusion method with Bacillus subtilis ATCC 6633 spores that were seeded into penicillin assay seed agar prepared in 1% phosphate buffer, pH 6. Incubation was at 30°. Standards for assay of serum were prepared in pooled human serum devoid of background antimicrobial activity. Assays were performed according to the method of Fare et al (5). The microbiological assay was linear between 0.75 and 5.0 µg/ml.

RESULTS AND DISCUSSION

Figure 2 shows a typical chromatogram of ceftizoxime and cefotaxime, these peaks were not disturbed by other serum components. Typical chromatogram of urine sample is shown in Figure 3; no interfering peaks can be observed although the sample was injected without prior clean up. The retention time was 6.0 min for ceftizoxime and 8.5 min for cefotaxime. As shown in Figure 4a and 4b, a linear relationship exists for known concentrations of ceftizoxime in serum and diluted urine respectively over a 2 to 200 µg/ml range as plotted against the corresponding peak height ratios. Linear regression analysis of the standard calibration

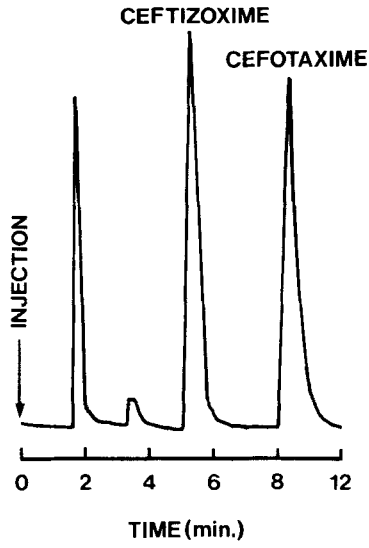

Fig. 2. Typical chromatogram for a serum sample containing 108.5 µg of ceftizoxime/ml

lines yielded the respective equations: $y = 0.010\ x - 0.021$ (r, 0.998) for serum, and $y = 0.023\ x - 0.031$ (r, 0.998) for urine. These correlations indicated an excellent linearity. The precision of the HPLC method was determined by assaying five replicate serum samples containing 60 and 120 µg/ml of ceftizoxime. Each sample was assayed in triplicate. The low coefficients of variation obtained (2.04 and 1.93%) show the precision of this HPLC method. Reproducibility data were obtained from five frozen serum samples containing 60 and 120 µg/ml of ceftizoxime and thawed on five different days; the coefficients of variation from day to day were 3.70 and 2.75% for both ceftizoxime concentrations. Three different ceftizoxime concentrations in pooled human serum and in phosphate buffer (pH, 7.4) were used to determine the precipitation recovery ratio. The protein precipitation recovery ratio was 1.04 ± .05 (mean ± S.D.).

Some antibiotics commonly used and probenecid were tested for interference with ceftizoxime and cefotaxime peaks; serum samples containing gentamicin (10 µg/ml), tobramycin (10 µg/ml) and probenecid (1,0 g given P.O. to healthy volunteer) (4) did not interfere with the analysis. To verify the application of this HPLC method to the serum

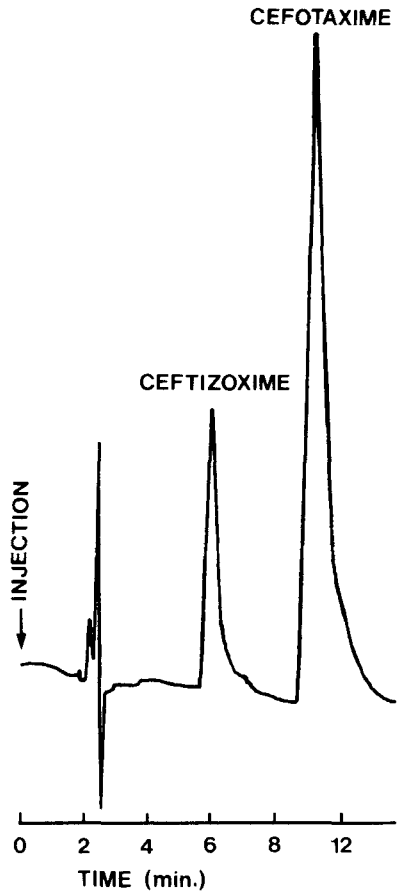

Fig. 3. Typical chromatogram for a urine sample containing 16.3 µg of ceftizoxime/ml.

determination of cefotaxime using ceftizoxime as an internal standard, expected concentrations of desacetylcefotaxime (the active metabolite of cefotaxime) were added to serum samples. The desacetyl metabolite separated very well, with a retention time of 3.5 min. However, ampicillin (20 µg/ml) would interfere with the assay of cefotaxime, with a retention time close to the desacetyl cefotaxime. The sensitivity limit of assay for serum samples was 2.5 µg/ml when a signal-to-noise ratio of 2 or greater was used as a criterion for a significant response. This is in contrast with the determination limit reported by Suzuki and

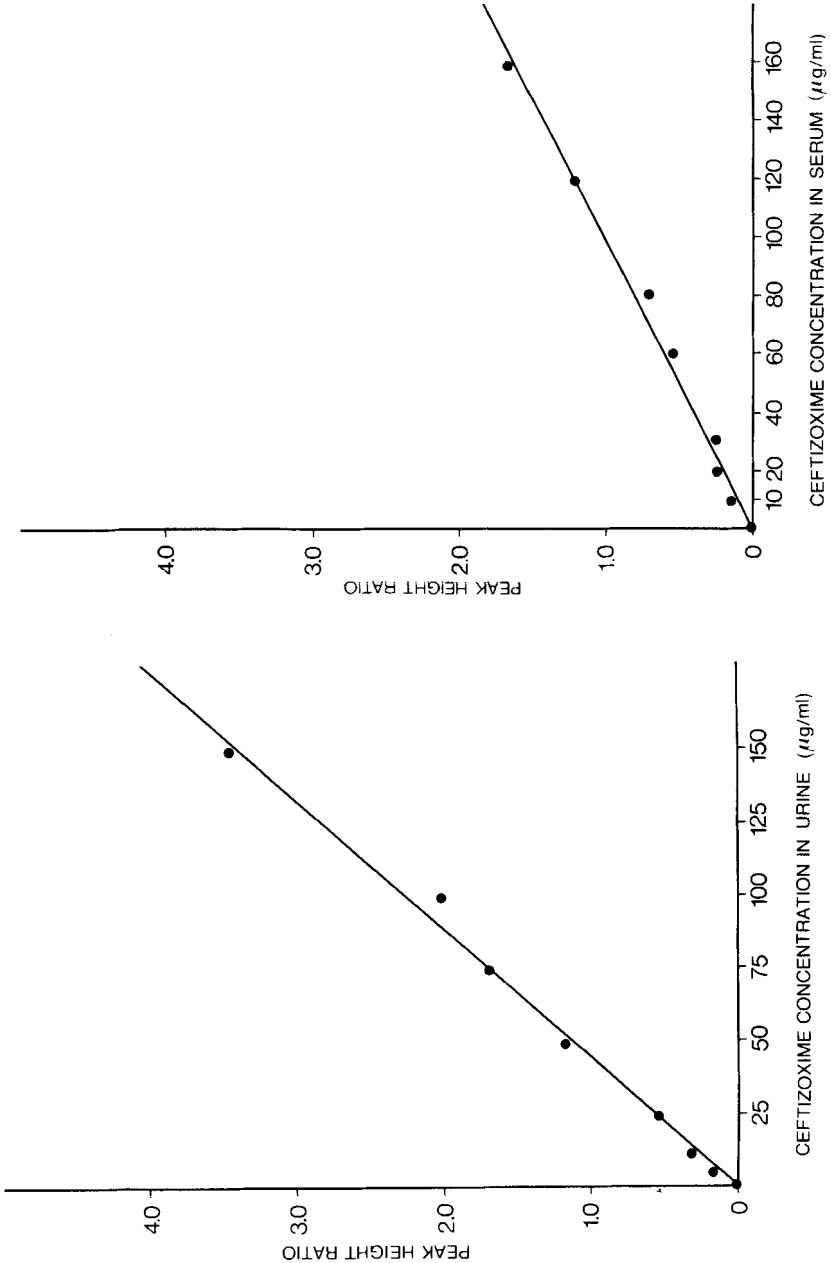

Fig. 4. Standard calibration curve of ceftizoxime in serum (a) and in diluted urine (b).

TABLE 1

Comparison Between HPLC Determination of Ceftizoxime and Microbiological Assay (25 Serum Samples)

		HPLC (μg/ml)	Microbiological assay (μg/ml)
pt 13, day 2	(0.25 h)	28.3	30.3
	(0.5 h)	43.4	38.8
	(0.75 h)	39.0	34.3
	(1.0 h)	38.6	39.2
	(1.5 h)	46.4	39.0
	(2.0 h)	33.9	31.1
	(3.0 h)	26.1	22.2
	(4.0 h)	17.7	14.9
	(6.0 h)	9.9	8.4
	(8.0 h)	6.6	6.1
	(10.0 h)	6.4	2.2
pt 3, day 1	(0.06 h)	177.1	205.1
	(0.25 h)	74.1	99.3
	(0.5 h)	57.4	56.3
pt 3, day 1	(0.75 h)	41.0	40.4
pt 2, day 1	(3.0 h)	14.6	11.3
	(10.0 h)	2.9	1.0
pt 10, day 1	(1.0 h)	36.2	27.0
	(1.5 h)	32.7	25.4
blank		0	0
blank		0	0
spiked plasma	(250 μg/ml)	248.2	226.6
" "	"	260.2	294.7
" "	(60 μg/ml)	60.5	59.6
" "	"	60.3	64.6

coworkers (0.2 μg/ml for serum at 0.01 A.U.F.S.) (1), but this may be explained by a larger dilution effect in the sample preparation (1.0 ml vs 0.3 ml) and a wider criterion for the evaluation of the limit of sensitivity.

The Table 1 shows the actual data obtained by microbiological assays. The comparison of these two methods yielded a correlation coefficient of 0.987. In the lower range of ceftizoxime serum concentrations, the microbiological assay showed slightly lower values than did the HPLC assay. The last ceftizoxime concentration used in constructing HPLC standard serum curve was 10 μg/ml; this may explain the lack of precision of the HPLC method at low ceftizoxime concentrations.

Although microbiological methods are more widely used they have their share of disadvantages. The HPLC assay generated results within 10-15 minutes after starting a limited number of samples; microbiological assays are more time consuming. When an antibiotic has active metabolite(s) or forms active decomposition products or when two antibiotics are administered, the highly specific HPLC methods are preferable(6-8).

The HPLC method described here may allow simultaneous determination of ceftizoxime and cefotaxime, which has distinct advantage over conventional methods for studies of comparative tissue penetration.

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